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#### [54] MOTILITY SCANNER AND METHOD

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[51] Int. Cl.<sup>4</sup> ...... G01N 21/00; G06K 9/00; C12M 1/34

[52] U.S. Cl. 356/442; 382/6; 435/291; 356/244; 350/525

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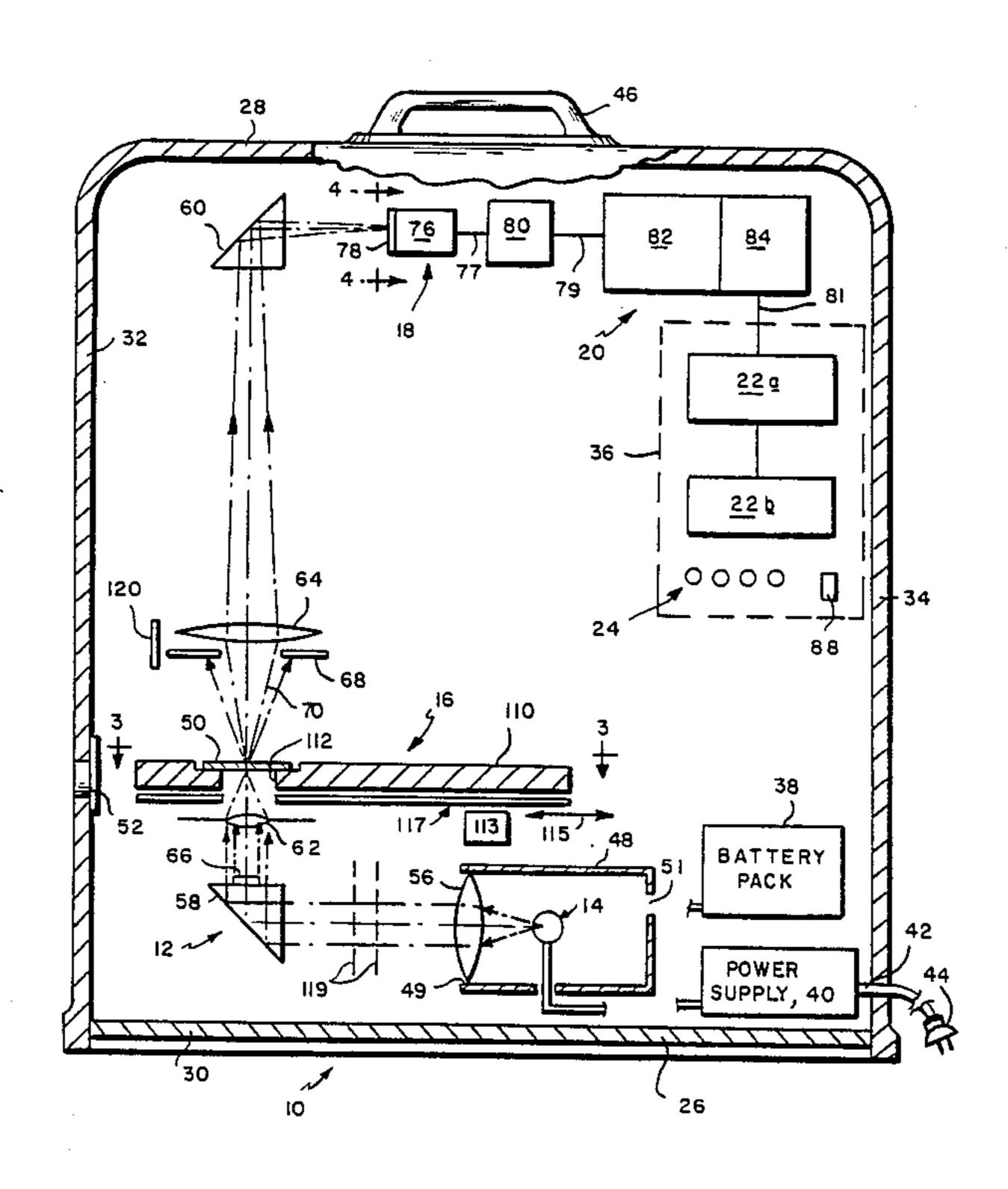
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Benson

#### ABSTRACT

A motility scanner and method are disclosed for characterizing the motion of sperm, bacteria, particles suspended in flowing fluids and the like. The motility scanner preferably is mounted as a portable unit in a container and includes an optical system, a source of illumination for the system, a heated specimen support disposed in the system, ratiation sensing means coupled to the system, signal processing means for analyzing signals generated by the radiation sensing means, and a front panel disposed on the container and featuring an output screen, a printer and controls required for operating the motility detector, including a power switch. A disposable specimen holder of unique design allows external loading thereof and its positioning on the heated specimen support. The source of illumination can be a conventional filament lamp or one or more LED's. Power for the motility scanner can be self contained by incorporating a battery pack within the container or it can be derived from a conventional 110 V.A.C. outside source via a cord and plug.

#### 15 Claims, 3 Drawing Sheets



526, 533, 536

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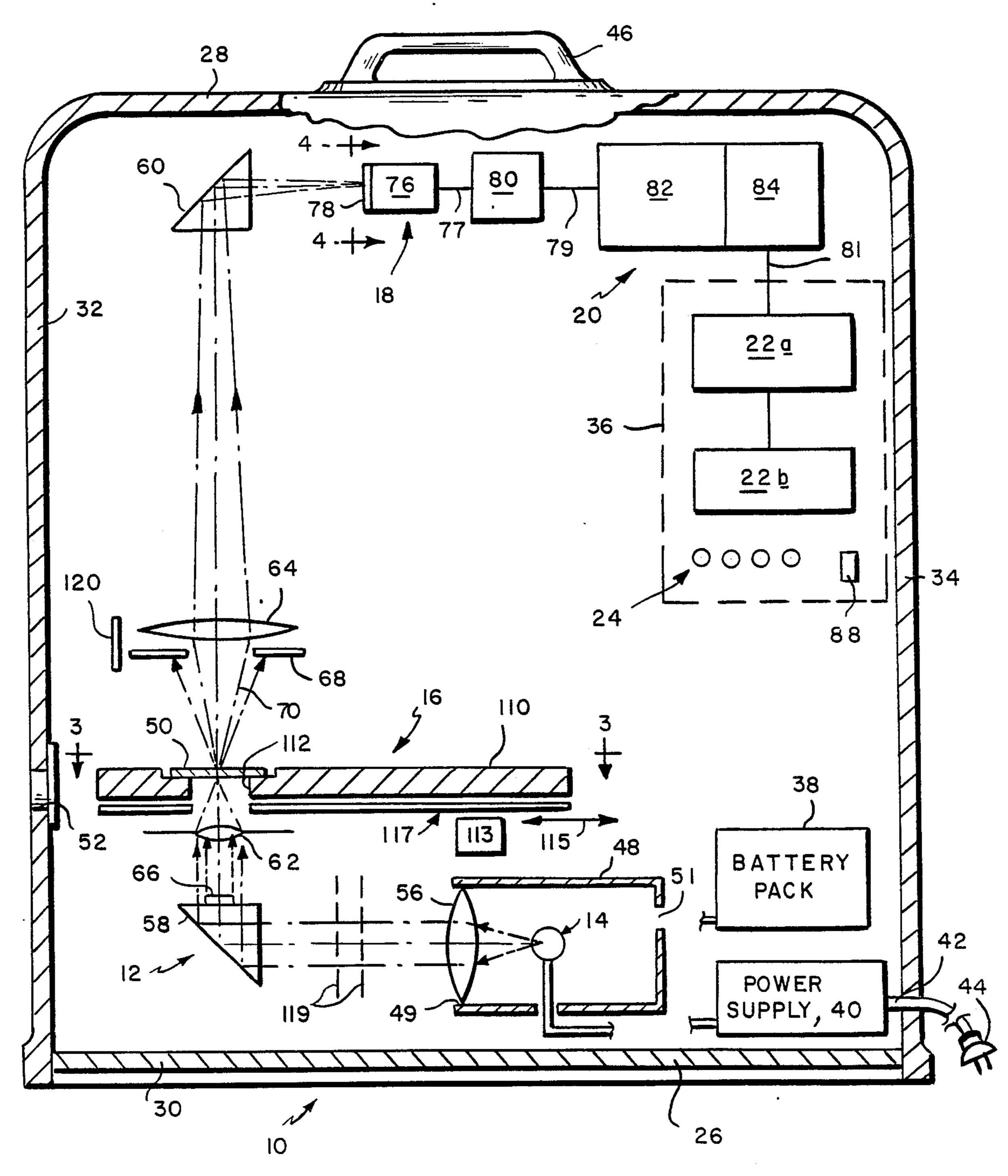
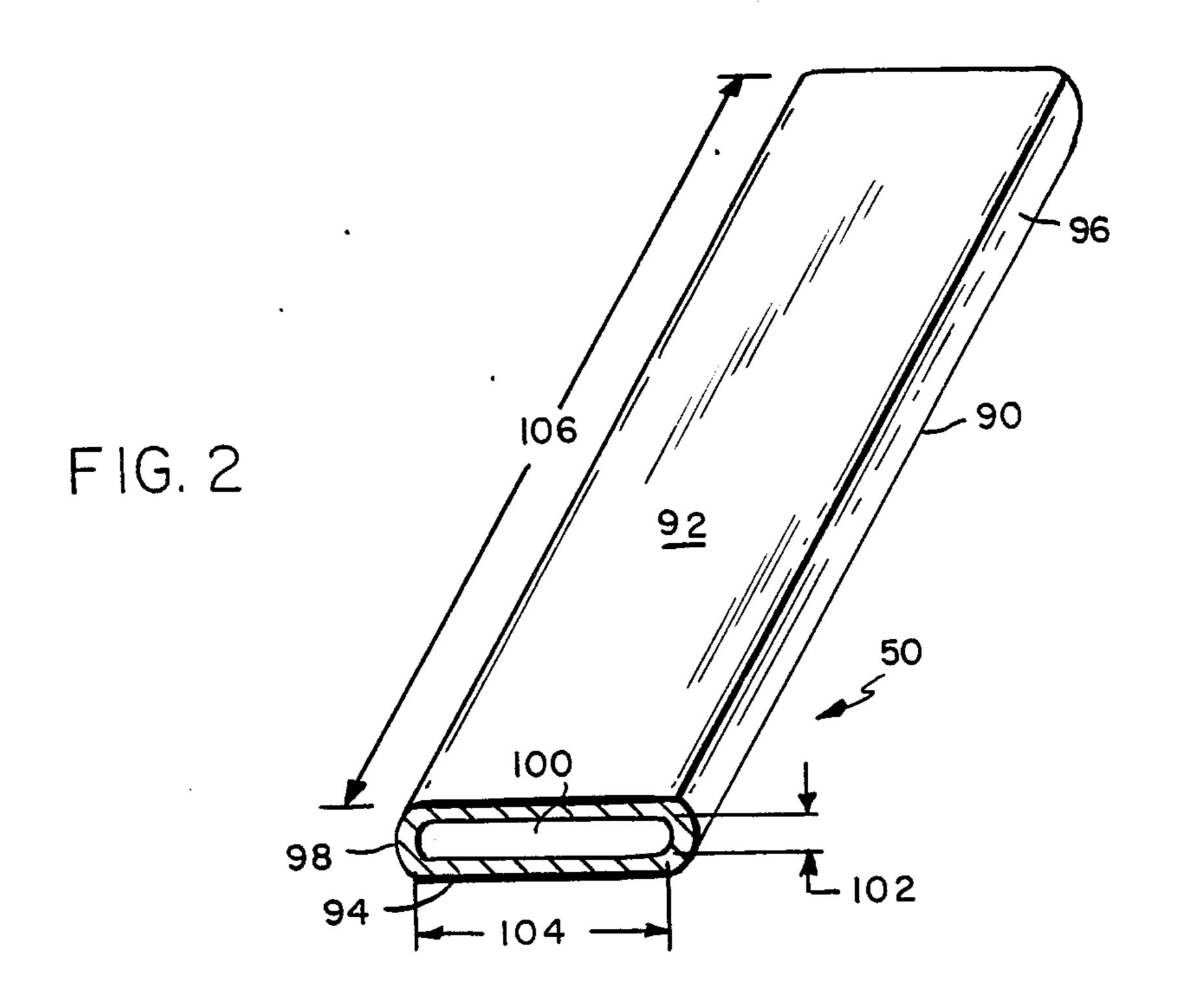
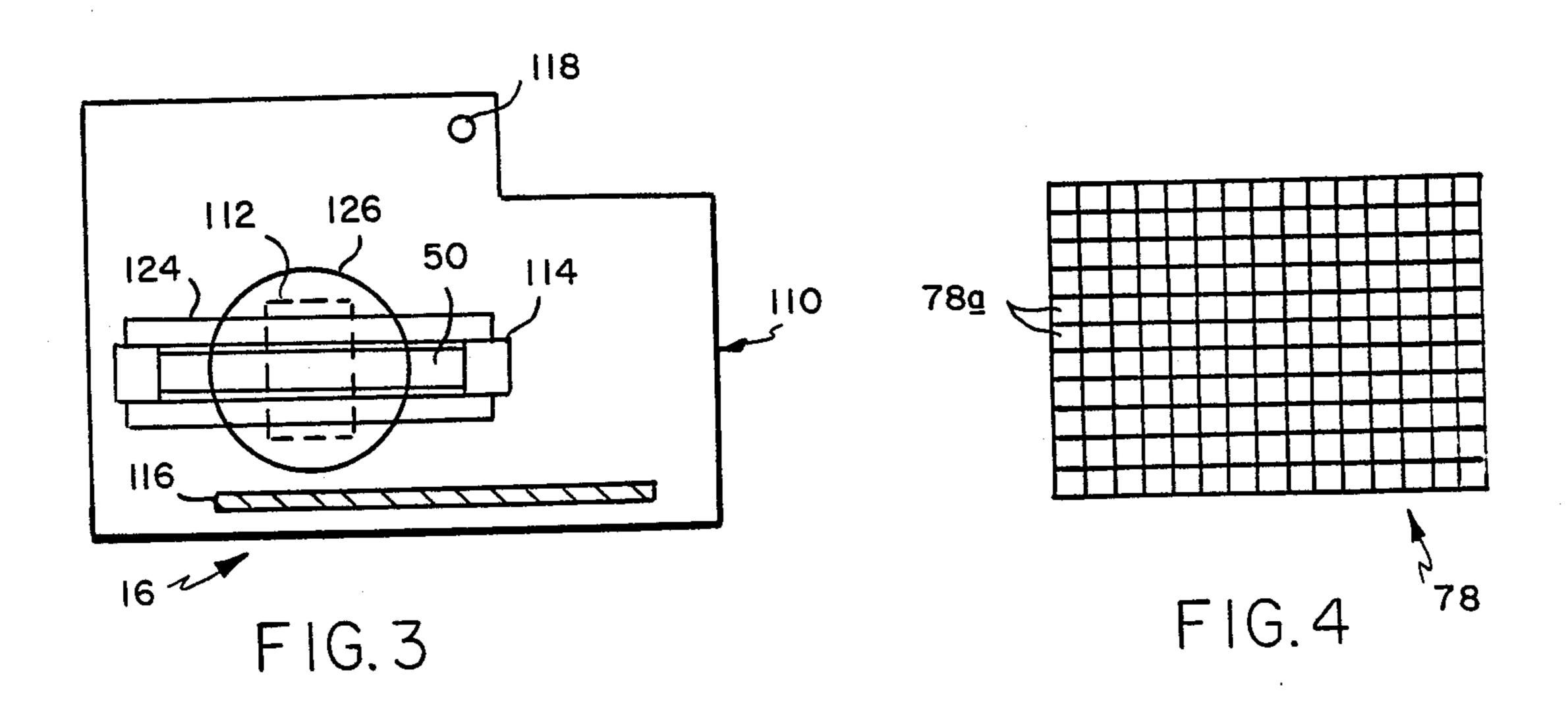
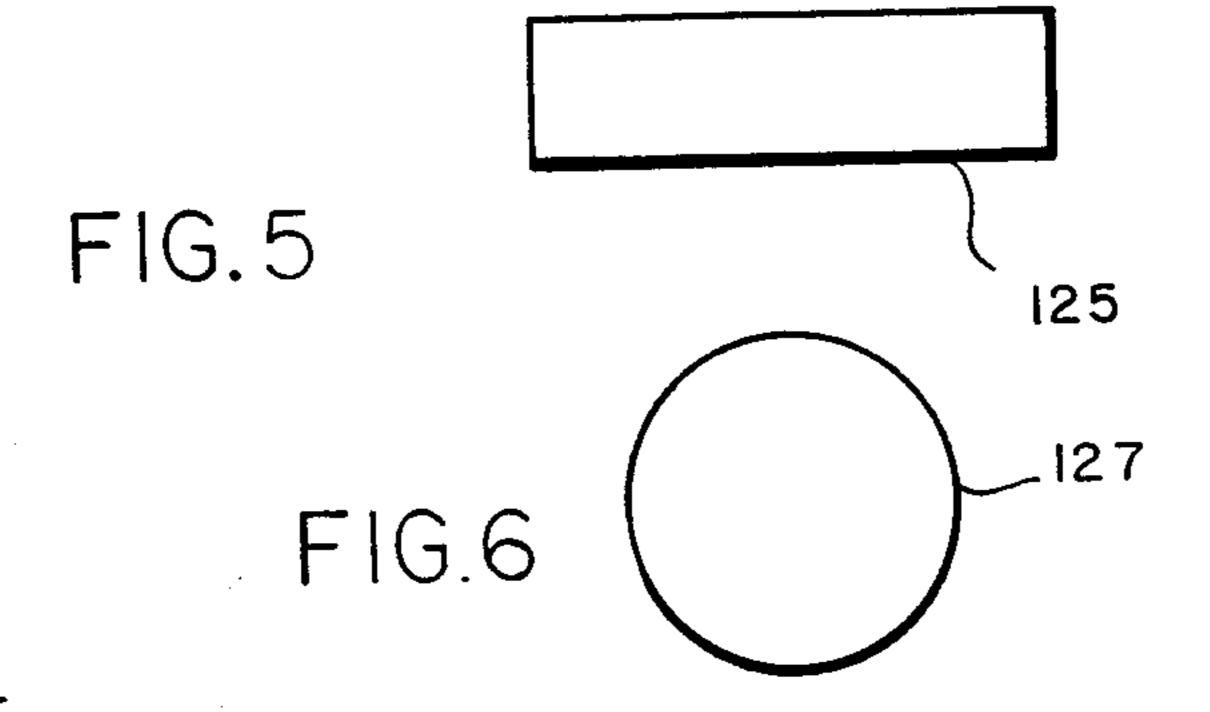


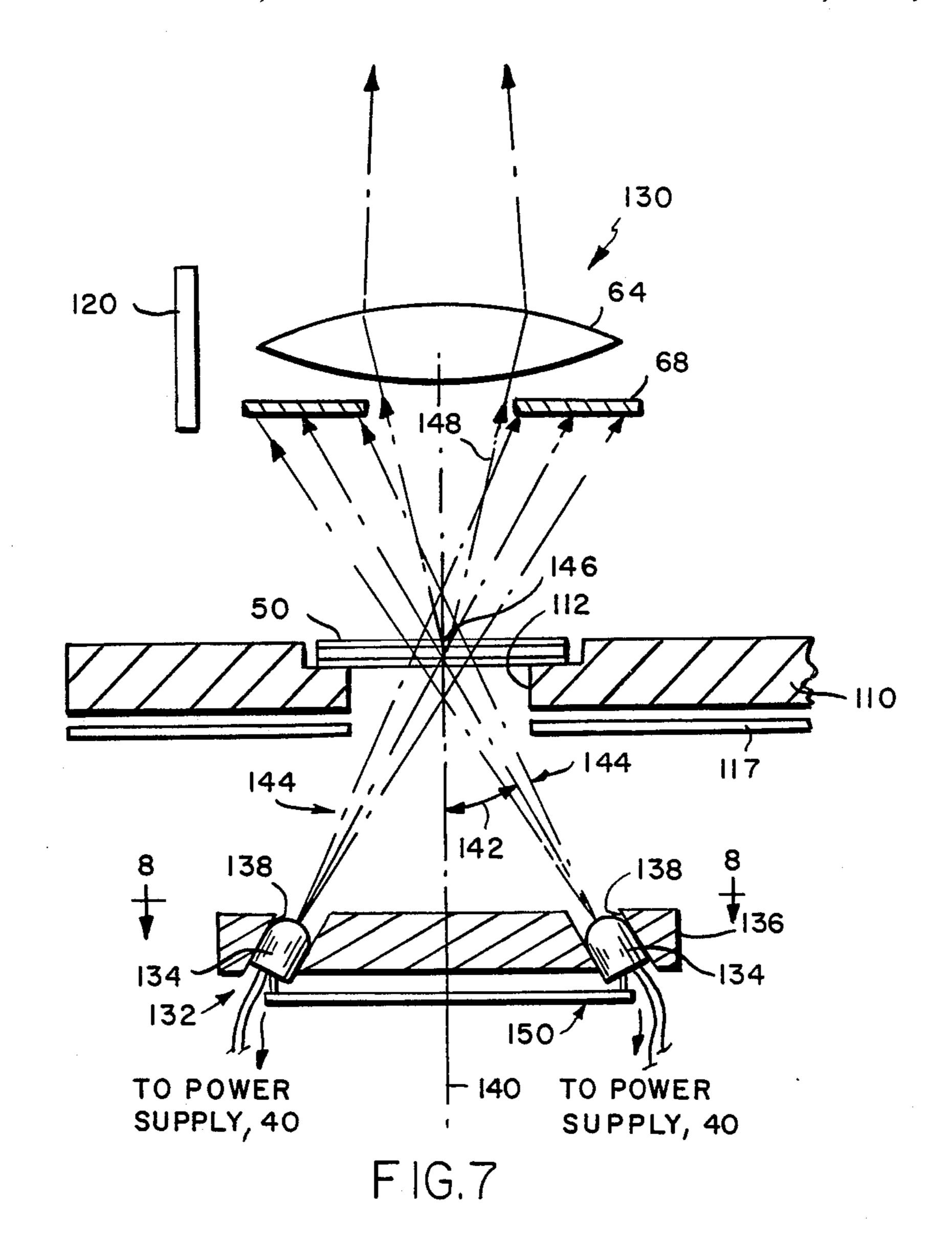
FIG. I

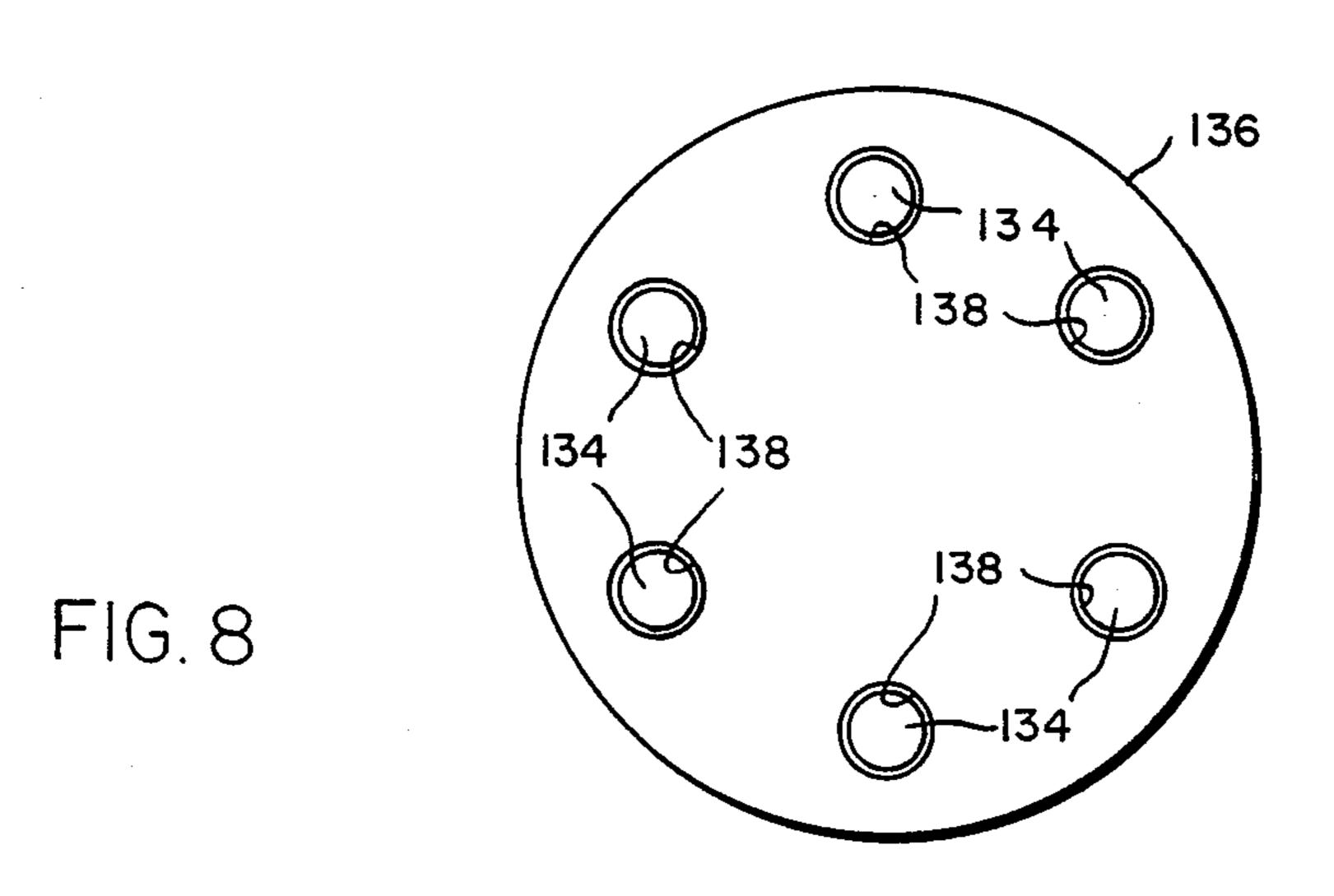
U.S. Patent











#### MOTILITY SCANNER AND METHOD

#### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

The present invention relates generally to the characterization of the motion of particles suspended in a liquid medium and, more particularly, to a device and a method developed for characterizing the motion of sperm, bacteria, particles suspended in flowing fluids, 10 Brownian motion and the like.

#### 2. The Prior Art

The characterization of the motion of particles suspended in a liquid medium is of particular significance in fertility analysis. The term "characterization" as used in this specification and in the appended claims is intended to define a procedure involving analyzing and determining the motion of particles in a liquid medium. Motility analysis as regards fertility includes the determination of sperm motility and mean sperm velocity. The term "sperm motility" is intended to to be defined as the fraction of sperm moving among all the sperms in a given specimen sample. The term "progressive motility" is intended to define the fraction of sperm moving in an approximately constant direction. The term "progressivity" or "linearity" is the ratio between the distance travelled and the track length.

Motility analysis is undertaken regularly for animals, such as horses, in particular race horses, and prime bulls, in order to establish and to keep a permanent <sup>30</sup> running record of the quality of their semen, hence their breeding potential. Motility analysis also represents an important segment in diagnosing certain reproductive problems in the human male.

For the most part, sperm motility and mean sperm 35 velocity are simply estimated by visual examination of a drop of semen on a slide. The results of such visual examinations vary widely, often by as much as 40%, from one observer to another. Further, one cannot estimate, purely on a visual examination, linearity or veloc- 40 ity distribution functions. In order to determine such linearity or velocity distribution functions, a method of multiple exposure time-lapse photography has been developed. This method is tedious and time consuming in that it requires the manual counting of the sperm 45 tracks, followed by manual derivation of the distributions of linearity and velocity. In order to speed up this manual method, a computerized version thereof has been developed which allows for the calculation of the distribution functions, but only after the sperm tracks 50 first have been manually outlined by using an interactive indicating device such as a light pen or a "mouse". A further improved version employs a microscope attached to a computer, video recorder and other peripheral items. This improved version is designed to analyze 55 a drop of semen in a special cell, called the Makler cell. The Makler cell is such that it maintains an exact narrow spacing, usually 0.01 mm, between its adjacent walls. Such an exact narrow spacing is required so as to provide a sharp focus for the microscope image, to 60 reduce the visual density and thus enable the computation of sperm density. The narrow spacing of the Makler cell, however, constricts the motion of the sperm tails. Sperm tails are believed to require 0.02 to 0.10 mm diameter about their axes, depending on species, in 65 order to operate and move freely, i.e., without constriction. Consequently, a system employing the narrow Makler-type cell spacing adversely affects the very

quantities, e.g., motility, velocity and linearity, that it is designed to measure. Such system also makes it very difficult to measure the motion of sperm in a diluent since sperm density is reduced to such a degree that obtaining statistically significant numbers of sperm requires a long time, and the superposition of many successive fields. The measurement of sperm motion in a diluent is frequently required in motility analysis. For example, in in vitro fertilization experiments, the semen may be diluted by a factor of 100 to 500. Further, the Makler cell is expensive to make and, in use, it is difficult to maintain at an exact, controllable temperature.

#### SUMMARY OF THE INVENTION

It is a principal object of the present invention to overcome the above disadvantages by providing an improved motility scanner and method for characterizing the motion of sperm, bacteria, particles suspended in flowing fluids and the like.

More specifically, it is an object of the present invention to provide a motility scanner and method for characterizing the motion of sperm, bacteria, particles suspended in flowing fluids and the like, comprising an optical system including an imaging lens having a useful depth of field at its object plane of at least about 0.2 mm; a source of illumination provided for the optical system; a heated specimen support disposed in the object plane of the imaging lens; a specimen holder, preferably disposable, designed to be positioned on the heated specimen support; radiation sensing means coupled to the optical system at the imaging plane of the imaging lens; signal processing means for analyzing the signals generated by the radiation sensing means; and display means connected to the signal processing means for displaying information characteristic of the motion of the sperm, bacteria, particles suspended in flowing fluids or the like. Preferably, the specimen holder comprises a flat tubular member formed of a hard transparent material, such as glass or plastic, and formed with a pair of spaced parallel sides defining a specimen holding channel therebetween. Preferably, this specimen holding channel has a depth of at least about 0.1 mm between the pair of its spaced parallel sides. The source of illumination can be a conventional filament lamp or it can comprise one or more LED's, preferably emitting infrared radiation. Preferably, the motility scanner is contained within a portable or transportable housing. A battery pack may also be disposed within the housing to provide a source of power for the illumination source. Alternatively, the motility scanner is provided with a transformer and a power cord whereby it can be connected to a conventional 115 V.A.C. power source.

Other objects of the present invention will in part be obvious and will in part appear hereinafter.

The invention accordingly comprises the motility scanner and method of the present disclosure, its components, parts and their interrelationships, the scope of which will be indicated in the appended claims.

### BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the nature and objects of the present invention, reference is to be made to the following detailed description, which is to be taken in connection with the accompanying drawings, wherein:

FIG. 1 is a schematic elevation, partly in section, of a motility scanner constructed in accordance with the present invention;

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FIG. 2 is an isometric perspective view, on an enlarged scale, of a part of the motility scanner shown in FIG. 1;

FIG. 3 is a plan view, along the line 3—3, of a portion of the motility scanner of FIG. 1;

FIG. 4 is a view, on an enlarged sale and along the line 4—4 of another part of the motility scanner shown in FIG. 1;

FIG. 5 is a plan view of an alternate specimen holder; FIG. 6 is a plan view of still another specimen holder;

FIG. 7 is a partial view, similar to FIG. 1, but showing another embodiment of the motility scanner; and

FIG. 8 is a plan view along the line 8—8 of FIG. 7.

# DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In general, the illustrated embodiment of a motility scanner 10, as depicted in schematic elevation and partly in section, is designed for the characterization of the motion of particles suspended in a liquid medium and has particular application to fertility analysis. In addition to motility analysis of sperm, the motility scanner 10 also is useful in analyzing and determining the motion of other items of interest, such as bacteria, particles suspended in flowing fluids, Browninan motion, and the like.

The motility scanner 10 essentially comprises an optical system 12, a source of illumination 14 for the optical system 12, a heated specimen support 16, radiation sensing means 18 coupled to the optical system 12, signal processing means 20 coupled to the radiation sensing means 18, display means 22 coupled to the signal processing means 20, and control means 24 for operating the motility scanner 10. Preferably, the motility scanner 35 10 is mounted within a portable housing 26, including a top wall 28, a bottom wall 30, and four side walls, two of which 32 and 34 are shown in FIG. 1. In the embodiment of FIGS. 7 and 8, the source of illumination comprises one or more LED's, as hereinbelow more fully 40 described.

Preferably, the display means 22 and the control means 24 are located on a panel 36 set into the front side wall, not shown. Preferably, the motility scanner 10 is self-contained in that it incorporates its own power 45 source 38 in the form of a battery pack. In the alternative, the motility scanner 10 also preferably includes a power supply 40 and a power cord 42, having a plug 44, connected thereto. The source of illumination 14 preferably is connected in parallel to both the battery pack 38 and the transformer 40. It is to be understood that whenever the plug 44 is plugged into a conventional power outlet of 115 V.A.C., the internal battery pack 38 is automatically disconnected from also supplying 55 power to the illumination source 14 and will remain so disconnected as long as the plug 44 remains plugged into the conventional power outlet. A convenient handle 46 is secured to the top wall 28, centrally thereof.

Specimen to be characterized by the motility scanner 60 10 is externally loaded into a specimen holder 50, which is then placed on a motorized and heated specimen support 16, which retracts to position the specimen at the imaging point. The heated specimen support 16 slides through a slot 52 formed in the side wall 32. A 65 flexible member 54 preferably is secured on the inside of the wall 32 so as to maintain the interior of the housing 26 dark.

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#### The Optical System

The optical system 12 of the motility scanner 10 comprises a collimating lens 56, a condensing lens 62 and an imaging lens 64. A pair of reflecting elements 58 and 60 may be included to bend the light path. Preferably, the reflecting element 58 comprises a fully reflecting prism, as shown, and element 60 comprises a front surface mirror. If desired, the element 58 also can be a mirror, or the like. It will be noted that the specimen support 16 and, more precisely, the thereon disposed specimen holder 50, are mounted at or near the focal plane of the condensing lens 62.

A focussing means 120 is operatively coupled to the imaging lens 64 to allow for the variation in the distance between the lens 64 and the specimen support 16. Also preferably one or more optical filters 119 are positioned between the collimating lens 56 and the reflecting element 58. These filters 119 are intended to remove unwanted spectral portions of the radiation emitted by the source 14.

The specimen holder 50 also is positioned during analysis at the object plane of the imaging lens 64, with the radiation sensing means 18 being disposed at the imaging plane of the imaging lens 64. An obscuring member 66, which preferably is a disk, is positioned on the exit side of the prism 58. It is to be understood that, in the alternative, the obscuring member 66 also can be bonded directly to the exit or entry sides of the prism 58. The function of the obscuring member 66, in cooperation with an aperture 68, is to assure that the imaging lens 64 receives no directly transmitted light. In the preferred embodiment, the condensing lens 62 produces an image of the obscuring member 66 on the aperture 68. Therefore, the imaging lens 64 accepts only light scattered or refracted into a cone of acceptance 70 by the specimen contained within the specimen holder 50. Consequently, any object present in the specimen which scatters or refracts light, such as a spermatozoon, will appear bright on a black background.

The imaging lens 64 is a single compound lens having an effective depth of field at its object plane of at least about 0.2 mm, and preferably about 0.5 mm, the significance of which will become apparent from below. The focal length of the imaging lens can vary between about 2.5 cm to about 5 cm, with the latter being preferred. The magnification ratio of the imaging lens 64 preferably is less than ten-to-one and, typically is about four-to-one. The imaging lens 64, for example, can be a "Schneider Componon" enlarger lens made by the Schneider Kreuznach company in Germany.

#### The Radiation Sensing Means

The radiation sensing means 18 of the motility scanner 10 preferably includes a light-sensitive device 76 provided with a plurality of light-sensitive cells 78, known as pixels, observe FIG. 4. The device 76, preferably a charge coupled device (CCD) or the like, generates an analog signal representative of the focused image on the pixels 78 of the device 78. Each pixel 78a is about 0.04 mm to about 0.05 mm in width. Since a typical sperm cell head has a diameter from about 0.002 mm to about 0.005 mm, one sperm cell head can be imaged on one pixel, using magnification 4 to 10. Further, each pixel 78a is designed so as to be able to discriminate, with uniform and stable sensitivity, up to sixty-four distinct levels of optical illumination intensity. Thus, light-scattering particles found in the speci-

men under investigation, such as spermatozoa, will appear bright in a dark field.

The analog signal representative of the focused image on the pixels 78 is transmitted via lead 77 to an analog to digital converter 80, also known as a frame grabber board, where the signal is converted to digital form on lead 79, and stored in RAM, preferably a 64 KByte RAM, from which it is available to the signal processing means 20. The signal processing means 20 essentially comprises a microprocessor 82 and a pre-programmed device 84. The microprocessor 82 preferably is capable of accessing in excess of 1.5 MByte RAM, includede in the system, while the device 84 contains a plurality of pre-programmed read-only memory (ROM) chips. The output of the signal processing means 20 is transmitted via lead 81 to the display means 22. Display means 22 basically includes a screen 22a and a printer 22b. The screen 22a is just like any computer screen, and can be black and white, or it can be in any desired color combination and capable of displaying information in both graphical and alphanumeric form. The printer 22b preferably is a hard copy printer designed to reproduce the information appearing on the screen 22a on paper upon command of the operator, effected by depressing one of 25 the control buttons of the control means 24. A power switch 88 allows the motility scanner 10 to be rendered operational.

#### The Specimen Holder

The specimen holder 50 for the motility scanner 10 is shown, in isometric perspective and on an enlarged scale, in FIG. 2, and its operative use is illustrated in FIG. 3. As noted, the specimen holder 50 comprises a flat tubular member 90 formed of a hard, transparent 35 material, such as glass or plastic, with a pair of wide spaced parallel sides 92 and 94 and opposed short, curved connecting sides 96 and 98, cooperately defining therebetween a specimen holding channel 100. Preferably, the specimen holding channel 100, which is open at 40 its respective ends, is formed with a depth 102 of about 0.2 mm between its wide parallel sides 92 and 94, a width 104 of about 2 mm between its curved connecting sides 96 and 98, and an axial length 106 of about 5 cm between its respective open ends.

The operative use of the specimen holder 50 is best described with reference to FIGS. 1 and 3. After the specimen holding channel 100 of the specimen holder 50 has been filled with a properly diluted sample, as more fully described below, the specimen holder 50 is 50 placed on the retractable heated specimen support 16, which slides through the slot 52 in the side wall 32. Specimen support 16 includes a flat member 110, provided with a window 112 and a longitudinal slot 114. Member 110 is designed to slide in and out of the slot 52, 55 as indicated by an arrow 115, with the aid of means 117. Means 117 preferably is a motorised means and slides with the aid of a small electric motor 113. Member 110 preferably is heated via an electrical resistance wire 116 further and wider slot 124 designed to accomodate a microscope slide 125, if that is chosen to contain the specimen. In addition, member 110 also has a circular slot 126 designed to accomodate a Makler-type cell 127, if that is chosen to contain the specimen. It should be 65 noted that each of these slots 114, 124 and 126 is so located as to center the respective specimen holder over the window 112.

When the microscope slide 125 is used to hold the specimen, the density of the sperm population therein is not known since the spacing between the slide 125 and its coverslip is not known. A user of the motility scanner 10 would find the employment of the Makler-type cell 127 particularly useful whenever more concentrated sperm dilutions were to be examined.

It should also be noted that the source of illumination 14 is completely enveloped, save for an opening 49 for the collimating lens 56, and an air cooling aperture 51, in a suitable enclosure 48.

#### The Embodiment of FIGS. 7 and 8

In the embodiment illustrated in FIGS. 7 and 8, a simplified optical system 130 is employed in the motility scanner due to a diffrent source of illumination 132 used. This source of illumination 132 comprises at least one, and preferably more LED's 134, mounted in a ring-like manner in a support member 136. The support member 136 is provided with a plurality of apertures 138 to accommodate the LED's 134. Each of these apertures 138 is formed at an angle 142, inclined with respect to a central axis 140 of the member 136. The LED's are then mounted in the apertures 138 at this inclined angle 142, which is the angle between the maximum emission of radiation from the LED's 134 and the axis 140. This angle of convergence 142 can range from about 15° to about 45°, and preferably is about 25°. Variation in the angle of convergence 142 will either enhance or or weaken the motility scanner's ability to detect sperm rotation within the specimen holder 50. This is so since sperm rotation causes the sperm cell's image to increase or to decrease in intensity. The amplitude of this intensity change is related to the angle of incidence of the illumination on the sperm cell within the specimen holder 50, and can be controlled by varying the angle of convergence 142 of the LED's 134 by means 150. Means 150 preferably varies this angle of convergence 142 for all LED's simultaneously and is controlled manually by adjusting one of the knobs on the front control panel 36.

Each LED 134 produces an intense, concentrated beam of forward directed radiation 144 directed at a point 146 of intersection of the specimen holder 50 and the central axis 140. Since each LED 134 independently illuminates the whole field of investigative interest of the specimen holder 50, one such LED 134 can suffice. In order to enhance the uniformity of illumination at the specimen holder 50 however, it is preferred that a ring of LED's 134 be employed. The number of LED's in the ring can vary anywhere from three to about twelve, with a ring of six LED's being preferred, as illustrated in FIG. 8. The geometry of the ring of LED's 134 is so designed as to prevent direct radiation from the LED's 134 from reaching and entering the imaging lens 64. Thus, the only radiation to reach and enter the imaging lens 64 is radiation within a cone of acceptance 148, which is radiation scattered by the particles within the specimen holder 50 whose motion the motility scanner embedded therein. Member 110 also is provided with a 60 is designed to characterize. These particles will, therefore, appear bright in a dark background.

> Although any type of LED's can be used in the structure described with refernce to FIG. 7, LED's emitting radiation in the infrared region are preferred. This is so since radiation in the infrared range, and particularly about and near 880 nanometers, propagates well through the optical glass frequently employed for making the specimen holder 50 and the optical train. Fur

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ther, the sensitivity of the CCD detector 78 at and near 880 nm, is high, yielding a clear image, with adequate brightness. Still further, by using the longer wavelengths of illuminating radiation about and near 880 nm, we find that these longer wavelengths are less subject to 5 scattering by smaller particles suspended in the diluent medium, whose presence otherwise might adversely affect the output results of the motility scanner. For example, when using a typical milk-based diluent medium for sperm analysis, one of the problems frequently 10 encountered consists in the milk particles obscuring the optical transmission through the specimen holder 50 and in increasing the amount of scattering by such milk particles. As a consequence, the definition with which sperm cells normally can be discerned is markedly re- 15 duced. By using IR radiation, the scattering from the sperm tails also is reduced, enhancing thereby the characterization of sperm head movement. Unwanted scattering of infrared radiation, when compared to visible light, is greatly reduced, and consequently the image 20 quality obtained is improved. At present, we prefer to use IR radiation emitting LED's, with an intensity half angle of 8° and having a peak emission at or near 883 nm, such as the GaAlAs LED's currently made by the Siemens Werke GmbH, of West Germany, e.g., Sie- 25 mens SFH 484. Further, we prefer to operate these LED's at about 50% to about 75% power levels only so as to markedly increase their operational lifetimes.

It will be observed that in the structure of FIGS. 7 and 8 employing the LED's 134, as above described, in 30 addition to the enhanced optical efficiency gained at a reduced generated heat (only about 2 watts heat as contrasted with about six times as much heat generated by a conventional hot filament lamp), both the collimating lens 56 and the condensing lens 62 are omitted, 35 together with the prism 58 and the thereon placed obscuring member 66.

## The Preferred Process of the Invention

The preferred process of employing the motility de-40 tector 10 of the invention for characterizing the motion of sperm, bacteria, particles suspended in flowing fluids and the like will be illustrated with specific reference to equine semen, specifically that of stallions.

A specimen sample, i.e., a drop of semen obtained 45 from a stallion, is preferably first diluted with a suitable diluent and in a predetermined ratio. A suitable diluent is Hepes transparent extender or Kenney's equine extender. The predetermined ratio of diluent to sample for stallions is in the range of about 50:1 to about 250:1, and 50 preferably is about 250:1. The diluted sample is drawn up by capillary action into the specimen holding channel 100 of a specimen holder 50, one end of which is immersed below the surface of the diluted semen. The specimen holder 50 containing the diluted sample speci- 55 men is then placed on the heated specimen support 16, which is then moved back through the slot 52 into the motility scanner 10. As mentioned, the dimensions of the specimen holder 50 are such that it will slide within the channel guide 114 so that a portion of the specimen 60 holder 50 will lie over the window 112 formed in the flat member 110, observe FIG. 3.

With the motility scanner 10 having been activated by turning the power switch 88 on, the flat member 110, preferably made of a thermally conductive material, 65 such as aluminum, is maintained at a temperature of about 37° C. by an embedded resistor 116, through which an electric current is passed. A thermal sensor

118 also is embedded in the member 110 and is used to control the current passing through the resistor 116, and thereby its temperature. Member 110 is in turn maintaining the diluted sample within the specimen holder 50 at about the same temperature, i.e., about or slightly above normal body temperature. Due to this temperature and the dilution of the sample in the specified ratio, a milieu for the analyzed sample is being maintained throughout the process of the invention, which milieu most closely resembles that environment through which the sperm migrate normally and naturally. By depressing one of the control buttons of the control means 24, the source of illumination 14 and the signal processing means 20 are actuated and analysis of the specimen sample is effected. Focussing means 120 is provided to vary the distance between the imaging lens 64 and the specimen holder 50, producing thereby a sharp image at the image plane.

Since the effective depth of field of the imaging lens 64 at its object plane, precisely where the specimen holder 50 is disposed, exceeds the depth 102 of the specimen holder 50, the entire specimen depth is sharply imaged on the light-sensitive cells 78 of the charge coupled device 76. Further, since the imaging lens 64 is designed to maximize the modulation transfer function across a wide angular field, a high edge resolution of sperm in the analyzed sample is obtained. Sperm within the sample specimen now appear as bright objects on a black background. Successive images produced on the light sensitive cells 78, being representative of the sperm moving through the diluted sample, are then transmitted to the frame grabber board typically at about five frames per second, although higher or lower rates of image acquisition also can be used. These transmitted images are now stored in the RAM thereof. After a plurality of images have been so stored in the RAM, namely at least about 5 and preferably about 20 to 30 stored images, the image taking process ceases and analysis of the stored images commences.

A frame subtraction procedure first is employed which removes all stationary objects, caused by the presence of dirt or the like either in the optical system 12 or within the specimen sample, from the process so that only the moving parts, e.g., live sperm, of the specimen sample are dealt with by the system. A background frame is constructed from at least one, typically three, of the recorded video frames, by comparing these video frames and retaining, pixel by pixel, the minimum of the three pixel values. The background frame constructed in this way is subtracted pixel by pixel, from each of the recorded vodeo frames. The resulting video images contain only the non-static component of the original images.

Following the removal of stationary objects from adversely affecting the process of motion characterization, analysis of the transmitted frames is then effected in and by the coded device 84, specifically by the plurality of ROM chips contained in the device 84. These ROM chips have previously been pre-programmed with instructions to analyse the transferred images from the light-sensitive cells 78 of the device 76 and to derive sperm velocity and linearity distribution functions therefrom. The motion characterization process includes analysis of the sperm tracks in which the velocity along the sperm track and the velocity between track end points are calculated, and the track linearity is derived as the ratio of the distance between the starting and the end points of the tracks to the total track length.

Mean values of velocity and linearity are then computed and these averages, as well as the distributions of number of sperm versus velocity and linearity are displayed on the screen 22a and, if desired, printed on a hard copy by the printer 22b. Alternately, the tracks 5 may be plotted on a plotter peripheral device. A typical screen display and/or information appearing on a copy from the printer 22b is illustrated in Example I.

Example I					
DATA SUMMARY:	(time and date)				
TOTAL OBJECTS	50 million/ml. (185)				
MOTILE OBJECTS	23 million/ml. (88)				
MOTILE/TOTAL	47.6 percent				
AVERAGE VELOCITY	32.5 microns/second				
AVERAGE P-VELOCITY	24.1 microns/second				
AVERAGE PROGRESS	73.4 percent				

P	ROGRESSIVITY DISTRIBUTION (PERCENT)	
00–10	0	
10-20	0	
20-30	***** 2	
30-40	**********	2
40-50	****** 3	_
50-60	***************************************	
60-70	************ 21	
70-80	************ 21	
80-90	******* 16	
90-100	************ 5	3

	VELOCITY DISTRUBITION (MICRONS/SEC)	
00-10	** 1	3:
10-20	*********	
20-30	******** 27	
30-40	************ 29	
40-50	****** 19	
5060	****** 3	
60-70	** 1	40
70-80	0	
80-90	0	
90-100	0	

Computer counting of sperm tracks then yields an accurate determination of live sperm density, since the total volume of specimen examined is known exactly from the optical magnification and the internal wall separation of the specimen holder 50. In order to estimate the total sperm density, the dead sperm cells are identified by selecting all background (non moving) objects whose optical brightness and linear dimensions fall within certain limits. These limits are defined as multiples of the mean values determined for all the moving cells. In this way, use of an absolute illumination calibration can be avoided. The motility is then determined as the ratio of the number of mobile objects to the total number of objects falling within the above limits in the field.

Utilization of the motility scanner 10, together with 60 the specimen holder 50, thus allows for accurate motion measurements of particles suspended in a fluid because: a/the particles enjoy complete freedom of motion without being constricted by walls;

b/ the design of the specimen holder 50 allows ample 65 time for effecting the motion characterization of the specimen sample before any danger of the sample drying out;

- c/ the optical system 12 allows for all of the spermatozoa or other moving particles contained within the specimen sample within the specimen holder 50 to be kept in clear focus throughout their movement within the depth 102 of the specimen holding channel 100;
- d/ the known wall spacing of the specimen holder 50 allows exact determination of the volume, hence the density, of the sample; and
- e/ the geometry of the specimen holder 50 minimizes problems of mass fluid flows which might otherwise distort the data.

Thus it has been shown and described an improved motility scanner 10 and method designed for characterizing the motion of sperm, bacteria, particles suspended in flowing fluids, Brownian motion and the like, which scanner 10 and method satisfy the objects and advantages set forth above.

Since certain changes may be made in the present disclosure without departing from the scope of the present invention, it is intended that all matter described in the foregoing specification or shown in the accompanying drawings, be interpreted in an illustrative and not in a limiting sense.

What is claimed is:

- 1. A motility scanner for characterizing the motion of sperm, bacteria and particles in fluid, comprising:
  - (a) an optical system including an imaging lens;
  - (b) a source of illumination for said system;
  - (c) a heated specimen support disposed in the object plane of said imaging lens, said heated specimen support designed to accommodate a specimen holder;
  - (d) radiation sensing means coupled to said system at the imaging plane of said imaging lens;
  - (e) signal processing means for analyzing signals generated by said radiation sensing means; and
  - (f) display means coupled to said signal processing means for displaying information characteristic of said motion;
  - (g) wherein said imaging lens has a depth of field at its object plane of about 0.2 mm and wherein said optical system further comprises a pair of reflecting elements, a collimating lens interposed between said source of illumination and one of said pair of reflecting elements, a condensing lens mounted between said one of said pair of reflecting elements and said specimen support, optical filters to remove unwanted portions of the radiation emitted by said source, and an aperture mounted between said specimen support and said imaging lens, said motility detector further including an obscuring member disposed on said one of said pair of reflecting elements.
- 2. The motility of claim 1 wherein said source of illumination comprises at least one LED.
- 3. The motility scanner of claim 2 wherein said LED is emitting in the infrared range and wherein said LED is directed at an angle so as to provide dark-field illumination of the specimen, wherein said angle ranges from about 15° to about 45°, and wherein said motility scanner is provided with means to vary said angle.
- 4. The motility scanner of claim 1 wherein said source of illumination is a filament lamp.
- 5. The motility scanner of claim 1 wherein said specimen holder is disposable and comprises a flat tube formed as a unitary structure of a hard, transparent material, said flat tube including two spaced parallel sides defining a specimen holding channel therebe-

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tween, said channel having a depth of about 0.05 to about 0.20 mm between said spaced parallel sides.

- 6. A motility scanner for characterizing the motion of sperm, bacteria and particles in fluid, comprising:
  - (a) an optical system including an imaging lens;
  - (b) a source of illumination for said system;
  - (c) a specimen support disposed in the object plane of said imaging lens;
  - (d) radiation sensing means coupled to said system at the imaging plane of said imaging lens;
  - (e) signal processing means for analyzing signals generated by said radiation sensing means; and
  - (f) display means coupled to said signal processing means for displaying information characteristic of said motion;
  - (g) wherein said specimen support is heated and is provided with slots to accommodate a specimen holder formed as a unitary structure of tubular shape for characterizing said motion of sperm, bacteria and particles in fluids within said specimen 20 holder designed for said scanner as well as a microscope slide and a cell which maintains an exact narrow spacing between its adjacent walls;
  - (h) and wherein said specimen holder is disposable;
  - (i) wherein said signal processing means comprises a 25 video frame grabber board including a plurality of ROMs and RAMs.
- 7. The motility scanner of claim 6 further including a portable housing, a source of power for said illumination source, a video display coupled to said frame grab- 30 ber board, a printer coupled to said video display, and control means for operating said motility detector.
- 8. A motility scanner for characterizing the motion of sperm, bacteria and particles in fluid, comprising:
  - (a) a portable housing having a bottom and a top wall 35 and four side walls, a front panel formed on one of said side walls, apertures formed in two opposed side walls of said four side walls, and having a battery pack mounted therein;
  - (b) an optical system mounted within said housing, 40 said system including a pair of reflecting elements, an imaging lens and a condensing lens mounted within said pair of reflecting elements, and a collimating lens;
  - (c) a source of illumination to direct a collimated 45 beam of light via said collimating lens at one of said pair of reflecting elements;
  - (d) a heated specimen support disposed in said object plane of said imaging lens;

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- (e) radiation sensing means mounted to receive a beam of light focused thereat by said imaging lens and reflected by the second of said pair of reflecting elements and generating signals representative of said received beam of light;
- (f) signal processing means coupled to said radiation sensing means for analyzing said signals generated by said radiation sensing means; and
- (g) display means coupled to said signal processing means for displaying said analyzed signals representative of said motion; and
- (h) an aperture mounted between said specimen support and said imaging lens and an obscuring member disposed on said one of said pair of reflecting elements, said obscuring members causing said optical system to provide a dark-field illumination of the specimen.
- 9. The motility scanner of claim 8 wherein said radiation sensing means includes a plurality of pixels, each of said plurality of pixels being about 40 to 50 microns in diameter and capable of discriminating about 64 levels of light intensity.
- 10. The motility scanner of claim 8 wherein said signal processing means includes a microprocessor, capable of accessing from about 1.0 MByte to about 4 MByte RAM and said display means includes both a video screen and a hard copy printer.
- 11. The motility scanner of claim 8 further including a disposable specimen holder designed to be positioned on said heated specimen support, said holder comprising a flat tube formed with a pair of spaced opposed parallel sides defining a specimen holding channel therebetween, said channel having a depth of about 0.2 mm between said spaced opposed parallel sides, said tube having a width of about 2 mm and a length of about 5 cm, said heated specimen support being provided with guide means for said specimen holder along its axial length.
- 12. The motility scanner of claim 11 wherein said disposable specimen holder is formed of glass or quartz.
- 13. The motility scanner of claim 11 wherein said disposable specimen holder is formed of plastic.
- 14. The motility scanner of claim 8 wherein said imaging lens has a magnification ratio of about four to one.
- 15. The motility scanner of claim 8 wherein said heated specimen support is designed to be slidingly moved in and out of one of said apertures with the aid of motorized transport means.

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